Journal of Horticultural Science & Biotechnology (2008) **83** (1) 43–50

Ultraviolet radiation effects on fruit surface respiration and chlorophyll fluorescence

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SUMMARY
High-value fruit crops are exposed to a range of environmental conditions that can reduce fruit quality. Solar injury (SI) or sunburn is a common disorder in tropical, sub-tropical, and temperate climates and is related to: 1) high fruit surface temperature; 2) high visible light intensity; and, 3) ultraviolet radiation (UV). Positional changes in fruit that are caused by increased weight or abrupt changes that result from Summer pruning, limb breakage, or other damage to the canopy can expose fruit to high solar radiation levels, increased fruit surface temperatures, and increased UV exposure that are higher than the conditions to which they are adapted. In our studies, we examined the effects of high fruit surface temperature, saturating photosynthetically-active radiation (PAR), and short-term UV exposure on chlorophyll fluorescence, respiration, and photosynthesis of fruit peel tissues from tropical and temperate fruit in a simulation of these acute environmental changes. All tropical fruits (citrus, macadamia, avocado, pineapple, and custard apple) and the apple cultivars 'Gala', 'Gold Rush', and 'Granny Smith' increased dark respiration (A_0) when exposed to UV, suggesting that UV repair mechanisms were induced. The maximum quantum efficiency of photosystem II (F_v/F_m) and the quantum efficiency of photosystem II (ΦII) were unaffected, indicating no adverse effects on photosystem II (PSII). In contrast, 'Braeburn' apple had a reduced F_v/F_m with no increase in A_0 on all sampling dates. There was a consistent pattern in all studies. When F_v/F_m was unaffected by UV treatment, A_0 increased significantly. Conversely, when F_v/F_m was reduced by UV treatment, then A_0 was unaffected. The pattern suggests that when UV repair mechanisms are effective, PSII is adequately protected, and that this protection occurs at the cost of higher respiration. However, when the UV repair mechanisms are ineffective, not only is PSII damaged, but there is additional short-term damage to the repair mechanisms, indicated by a lack of respiration to provide energy.

High-value fruit crops are exposed to a range of environmental conditions that can reduce fruit quality, while markets demand an almost perfect fruit appearance. Solar injury (SI) or sunburn is a common disorder in tropical, sub-tropical, and temperate climates. There are three general environmental factors related to SI: 1) high fruit surface temperature; 2) high visible light intensity; and, 3) ultraviolet radiation (UV; UV-A 320-400 nm; UV-B 280-320 nm; Woolf and Ferguson, 2000; Wünsche *et al*., 2001; Schrader *et al*., 2001). Fruits of different species respond differently to these three environmental factors (reviewed in Glenn *et al*., 2002). Tropical regions experience higher levels of UV radiation than do temperate zones at higher latitudes, because of the small solar zenith angle and the thinner stratospheric ozone layer (Krause *et al*., 1999; Madronich *et al*., 1998) and, depending upon the season, can have long periods of high temperatures and clear skies that result in SI damage. UV-B radiation damages PS II, and the effects can be measured through a reduction in variable chlorophyll fluorescence (Jansen *et al*., 1998). UV-A radiation causes increased formation of reactive molecules and decreased electron transport efficiency (White and Jahnke, 2002).

Plant mechanisms to protect tissues from SI damage are based primarily on secondary pigment development (Demmig-Adams and Adams, 1992), including increased synthesis of flavonoids (Solovchenko and Schmitz-Eiberger, 2003) and carotenoids. However, anthocyanins are not effective secondary pigment protectants at low and moderate levels (Solovchenko and Schmitz-Eiberger, 2003; Woodall and Steward, 1998). Early degradation of chlorophyll and reduced chlorophyll fluorescence are indicators of SI damage in fruit (Wünsche *et al*., 2001). Because mature and chlorophyll-free peppers and cucumbers did not respond by developing SI damage under high UV light, Rabinowitch *et al*. (1983; 1986) reasoned that chlorophyll, and its

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subsequent degradation, was also a causal agent of SI. Fruit, unlike leaves, generally lose chlorophyll during the maturation process, concomitant with an increase in the concentration of anthocyanins and carotenoids (Reay and Lancaster, 2001; Reay *et al*., 1998). However, Cheng (L. Cheng, personal communication) noted decreased carotenoid and xanthophyll cycle pigment levels as apple fruit developed. The development of anthocyanins (Chalmers and Faragher, 1977a,b) and carotenoids (Solovchenko and Schmitz-Eiberger, 2003), xanthophyll cycle pigments (Krause *et al*., 1999), and flavonoids (Reay and Lancaster, 2001) increased with increasing photosynthetically-active radiation (PAR) and UV radiation. In contrast, high temperatures reduced their development (Saure, 1990).

Positional changes in fruit caused by increased weight or abrupt changes due to Summer pruning, limb breakage, defoliation by insects, or other damage can expose fruit to higher levels of solar radiation, increased fruit surface temperatures, and increased UV exposure, higher than those to which they are adapted. These acute changes in environmental conditions can result in SI damage in all climates. To understand the effect of environmental factors on SI, we examined the short-term effect of a high fruit surface temperature (40ºC), saturating PAR (1,000 µmol m^{-2} s^{-1}), and short-term UV-A (19.5 W m^{-2}; from 320 – 400 nm) exposure on chlorophyll fluorescence, respiration, and photosynthesis in fruit peel tissues of tropical and temperate fruits, in a simulation of these acute environmental changes.

MATERIALS AND METHODS

Tropical fruit studies.

The species sampled were: 1) citrus [*Citrus sinensis* (L.) Osb., cv. Valencia]; 2) macadamia (*Macadamia integrifolia* Maiden and Betche, cv. 847); 3) avocado (*Persea americana* Mill., cv. Hass); 4) pineapple (*Ananas comosus* Merr., cv. Smooth Cayenne); and 5) custard apple (*Annona* spp. hybrid, cv. African Pride). All fruit were mature and approaching harvest when sampled in March 2004 at the Maroochy Research Station, Nambour, Queensland, Australia (26.4ºS; 152.6ºE; 31 m a.s.l.).

Peel samples were collected from fruit tissues that were oriented toward the sun, but not fully exposed because of canopy coverage. A 1 cm^2 core of fruit peel and flesh was extracted from each fruit. The length of the core was cut to 3 – 5 mm. The core was placed on a glass slide, and the base and exposed edges were coated with silicone grease to prevent gas exchange from surfaces other than the peel. The peel core was then placed inside the cuvette of a photosynthesis system (CIRAS-2; PP Systems, Amesbury, MA, USA). The cuvette CO_2 concentration was 500 µl l^{-1}. The cuvette had two ports oriented to the centre of the cuvette: 1) an entry for the chlorophyll fluorescence probe (FMS2 Hansatech; PP Systems); and, 2) an entry for a fibre optic probe from a UV source (Model LC5; Hamamatsu Inc., Middlesex, NJ, USA). Two optical filters were installed in the path of the radiation from the UV source: 1) a Hoya U-340 25 mm square (NT46-084; Edmund Optics Inc., Barrington, NJ, USA), which had a peak transmission at 340 nm and excluded visible and infrared wavelengths; and, 2) a UV transmission filter that passed approx. 85% of radiation less than 400 nm, but blocked 400 – 700 nm (A7028-03; Hamamatsu Inc.). The UV source provided 19.5 W m^{-2} from 320 – 400 nm, or about 50 – 60% of the natural incoming UV-A intensity at solar noon (30 – 40 W m^{-2}; Krause *et al*., 1999). The UV source had a UV-A:UV-B ratio of 26:1, while natural sunlight is approx. 20:1 (Krause *et al*., 1999). Radiation intensity from 200 – 290 nm was 0.5 W m^{-2}. The cuvette temperature was 40˚C. PAR was either 0 or 1,000 µmol m^{-2} s^{-1}, and the UV source was alternately "off" and "on" at both PAR levels. Peel samples were placed in the centre of the cuvette and brought to 40ºC in the dark, without UV, for 10 min equilibration. Following temperature and light equilibration, gas exchange and chlorophyll fluorescence were measured every 7 min. At PAR = 0, net photosynthesis (A_0) was measured and interpreted as dark respiration, and the maximum quantum efficiency of photosystem II (F_v/F_m) was measured.

The treatment sequence was: 1) PAR = 0, UV = off; 2) PAR = 0, UV = on; 3) PAR = 0, UV = off; 4) PAR = 0, UV = on. PAR was then set at 1,000 µmol m^{-2} s^{-1} for 10 min at 40˚C. At PAR = 1,000, net photosynthesis ($A_{1,000}$) was measured and the quantum efficiency of photosystem II (ΦII) was measured under saturating light conditions.

The next treatment sequence was: 1) PAR = 1,000, UV = off; 2) PAR = 1,000, UV = on; 3) PAR = 1,000, UV = off; and 4) PAR = 1,000, UV = on. Six replicates of each fruit were measured. Data were statistically analysed by species and by PAR level to test the effect of UV treatment at each PAR level, pooled over the alternate UV_{off} and UV_{on} conditions. The difference in response (UV_{off} minus UV_{on}) for A_0, $A_{1,000}$, F_v/F_m, and ΦII (respectively indicated as ΔA_0, $\Delta A_{1,000}$, $\Delta F_v/F_m$, and $\Delta \Phi II$) was tested against a value of 0 with a *t*-test ($P = 0.05$) at each PAR level.

Apple studies

Study 1: 'Braeburn' apple [*Malus sylvestris* (L.) Mill. var. *domestica* (Borkh.) Mansf.] on M.9 rootstock was grown in a 30 l container for 3 years at Palmerston North, New Zealand (40.4ºS; 175º.6 E; 45 m a.s.l.). After petal fall in 2003, eight plants were placed beneath a polycarbonate structure that excluded 98% of UV radiation, but maintained ambient environmental conditions and transmitted 96% PAR. UV and PAR transmission through the UV filter were measured on 20 March 2004 with a spectral radiometer (Model EPP 2000; StellarNet Inc., Tampa, FL, USA). Measurements were made at solar noon under clear sky conditions.

Eight plants were maintained nearby under ambient conditions (i.e., without a UV filter). Within the UV filter treatments, four trees were treated with a 3% reflective kaolin spray (PF) every 2 weeks (Surround WP Crop Protectant; Engelhard Corp., Iselin, NJ, USA). There were approx. 40 fruit on each tree. Thirty days before maturity, two trees of each treatment were selected and the fruit used in subsequent studies. Trees were selected based on uniformity of cropping and vegetative growth.

Baseline data were collected on 26 and 27 March 2004. Peel samples from the exposed shoulders of fruit were measured, as in the tropical fruit studies, for A_0, $A_{1,000}$, F_v/F_m, and ΦII. The residue from the PF treatment was

removed before measurement. In addition, the chlorophyll *a* and *b* (Chl), anthocyanin, and carotenoid concentrations of fruit, at the site of sampling, were estimated from reflectance measurements, according to Merzlyak *et al*. (2003). Reflectance was measured with a spectral radiometer (Model EPP 2000; StellarNet Inc.). Data for A_0, $A_{1,000}$, F_v/F_m, and ΦII were analysed in a split-plot design with the UV filter as the main plot, and PF treatment as the sub-plot, with six replicates of individual fruit, pooled over alternating UV_{off} and UV_{on} cycles at each PAR level. The difference in response (UV_{off} minus UV_{on}) for ΔA_0, $\Delta A_{1,000}$, $\Delta F_v/F_m$, and $\Delta\Phi II$ was tested against a value of 0 with a *t*-test ($P = 0.05$) at each UV filter and PF treatment level, for each PAR level.

Four trees, one representing each treatment, were placed in growth chambers under either 24ºC day/19ºC night, or 40ºC day/19ºC night conditions (New Zealand Controlled Environmental Laboratory, Palmerston North, New Zealand). The vapour pressure deficit was 1.0 kPa for all temperatures, the CO_2 concentration was 500 µl l^{-1}, and PAR was 1,350 µmol m^{-2} s^{-1} with a 10 h photoperiod. Illumination provided 12 W m^{-2} of UV from 300 – 400 nm.

Fruits were collected from the trees in the growth chambers after 5 d and 10 d and measured in a manner similar to that used in the baseline studies.

Data for A_0, $A_{1,000}$, F_v/F_m, and ΦII were analysed in a split-split plot design with four individual fruit replications. The main plot was the growth chamber day temperature, the sub-plot was the UV filter, and the split-split plot was the PF treatment, pooled over the alternate UV_{off} and UV_{on} cycles at each PAR level. The difference in response (UV_{off} minus UV_{on}) for ΔA_0, $\Delta A_{1,000}$, $\Delta F_v/F_m$, and $\Delta\Phi II$ was tested against a value of 0 with a *t*-test ($P = 0.05$) at each chamber temperature, UV filter, and PF treatment level, for each PAR level. Mean separation used a protected Duncan's multiple range test ($P = 0.05$) at each sampling date.

Study 2: 'Gala' apple on M.9 rootstock were planted in 1999 at 1 m × 6 m spacing in a North-South orientation at the USDA-ARS-Appalachian Fruit Research Station, Kearneysville, WV (39.4 N; 77.9 W; 169 m a.s.l.). In 2004, UV filters made from polycarbonate frames (5.0 m × 2.4 m × 0.8 mm) were constructed and placed at 60º and 120º angles adjacent to the trees. This arrangement of two panels on either side of the trees resulted in a pyramidal-shaped enclosure with a 0.5 m opening at the top and a 2.0 m opening at the bottom. Five trees were contained within each enclosure. The end of the frame within the tree row was left open. The panels were left on the trees from petal fall until harvest, and removed for a 24 hr period every 2 weeks for pest control application. The experimental design consisted of three treatments: 1) untreated control; 2) enclosure in the UV filter frames; and, 3) application of 3% kaolin spray (PF) every 2 weeks from petal fall until harvest, in a randomised complete block design with four replications of four trees per plot. Trees were hand-thinned to approx. 100 fruit per tree. Two representative fruit from each plot were collected on 21 June, 27 July, and 26 August 2004 from the three central trees per plot. Fruit peel samples from the exposed side of each fruit were measured for A_0, $A_{1,000}$, F_v/F_m, ΦII, and pigments (Chl, anthocyanins, and carotenoids) by reflection, as described above. After the data from the peel samples had been collected, the attached pulp was removed and the peel was frozen in liquid nitrogen. Chlorophyll *a* and *b* and xanthophyll pigments (violaxanthin, antheraxanthin, and zeaxanthin; VAZ) from two fruit peel discs (total area, 2 cm^2) per plot were measured by a procedure described in Cheng (2003).

Data for A_0, $A_{1,000}$, F_v/F_m, and ΦII were analysed in a randomised complete block design with three blocks in which two fruit were sampled from each plot, and the data pooled over the alternating UV_{off} and UV_{on} cycles at each PAR level. The difference in response (UV_{off} minus UV_{on}) for ΔA_0, $\Delta A_{1,000}$, $\Delta F_v/F_m$, and $\Delta\Phi II$ was tested against a value of 0 with a *t*-test ($P = 0.05$) at each treatment level, at each PAR level. Pigment concentration was analysed in a randomised complete block design with three blocks, pooling data from the two fruit samples. Mean separation used a protected Duncan's multiple range test ($P = 0.05$) at each sampling date.

In 2004, air temperature, PAR, and UV radiation within the enclosures, and in adjacent untreated trees, were measured from 3 – 5 September. Five thermocouples were installed within a single enclosure: two at 0.5 m and two at 3 m height, with one thermocouple on the East side of the canopy, and one on the West side of the canopy. Thermocouples were located at a depth of 20 – 30 cm within the enclosure. Air temperature above the enclosure was measured at 3.5 m above ground-level with a shaded thermocouple. A similar pattern of instrumentation was established in an adjacent, untreated plot without the enclosure. Data were collected every 5 s, and an hourly average computed. PAR and UV radiation were measured in each plot with two spectral radiometers (Model EPP 2000; StellarNet Inc.) on 3 – 5 September. Data were measured every hour. The two radiation sensors were calibrated against a known source. The sensor was positioned between two trees at a height of 1 m in each plot area.

Study 3: 'Granny Smith' and 'Gold Rush' apples planted in 1998 on M. 26 rootstock at a 2 m × 5 m spacing at the USDA-ARS-AFRS were sampled on 27 November 2004. Fruit peel samples from the exposed side of each fruit were measured for A_0, $A_{1,000}$, F_v/F_m, and ΦII, as described. Four replicates of each fruit were measured. Data were analysed by PAR level to test the effect of UV treatment at each PAR level, pooled over the alternating UV_{off} and UV_{on} cycles. The difference in response (UV_{off} minus UV_{on}) for ΔA_0, $\Delta A_{1,000}$, $\Delta F_v/F_m$, and $\Delta\Phi II$ was tested against a value of 0 with a *t*-test ($P = 0.05$) at each PAR level.

RESULTS

In apple Study 2 with 'Gala', the polycarbonate transmitted 95% of PAR and excluded 97% of UV radiation under the test conditions; however, in the field, 100% of PAR was measured within the enclosure, and UV was reduced by 75% daily (Figure 1). Midday temperatures were increased by 3º – 4ºC at 2 m height on sunny days, and less than 1ºC on cloudy days (Figure 2). At 0.5 m height, air temperature was increased 1º – 3ºC at

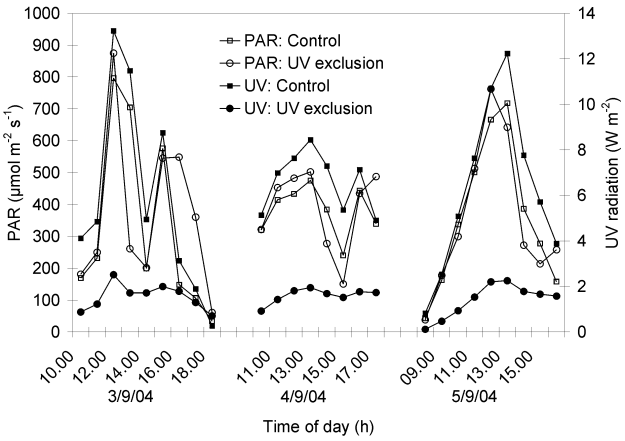

FIG. 1
Photosynthetically active radiation (PAR) and ultraviolet radiation (UV) in the ultraviolet exclusion chambers, and under ambient conditions, in an apple orchard between 3 – 5 September 2004 (Apple Study 2).

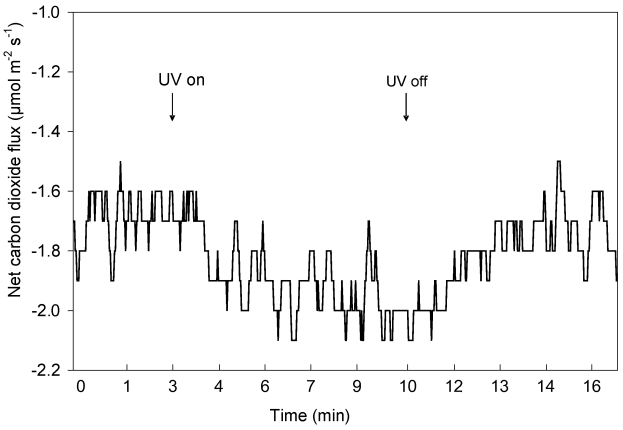

FIG. 3
Example of the net CO_2 flux (i.e., the difference between incoming and outgoing concentrations) response of apple peel to ultraviolet radiation.

midday on sunny days, and approx. 1°C on a cloudy day.

The UV treatment increased respiration (Figure 3), resulting in a more negative value when comparing CO_2 assimilation with UV_{off} minus UV_{on}. Leaf temperature increased by 0.1ºC when the UV lamp was used. The response of fruit peel to UV radiation was reversible. This example illustrates a minimal, but significant, response ($\Delta A_0 = 0.2$) in apple.

In all studies $A_{1,000}$, ΦII, $\Delta A_{1,000}$, and $\Delta \Phi II$ were unaffected by the treatments (data not shown; $P = 0.05$).

Chlorophyll *a* and *b* concentrations, measured by reflectance, correlated with chemical analysis ($y = 0.87x - 2.08$; $r^2 = 0.85$, $P = 0.05$). Anthocyanin and carotenoid concentrations measured by reflectance were not chemically validated.

Tropical fruit study

At PAR = 0, the presence of UV radiation increased the production of CO_2 in all species (Table I). The increased production of CO_2 (more negative value) was interpreted as increased dark respiration (A_0). $\Delta F_v/F_m$ was unaffected by the presence of UV radiation.

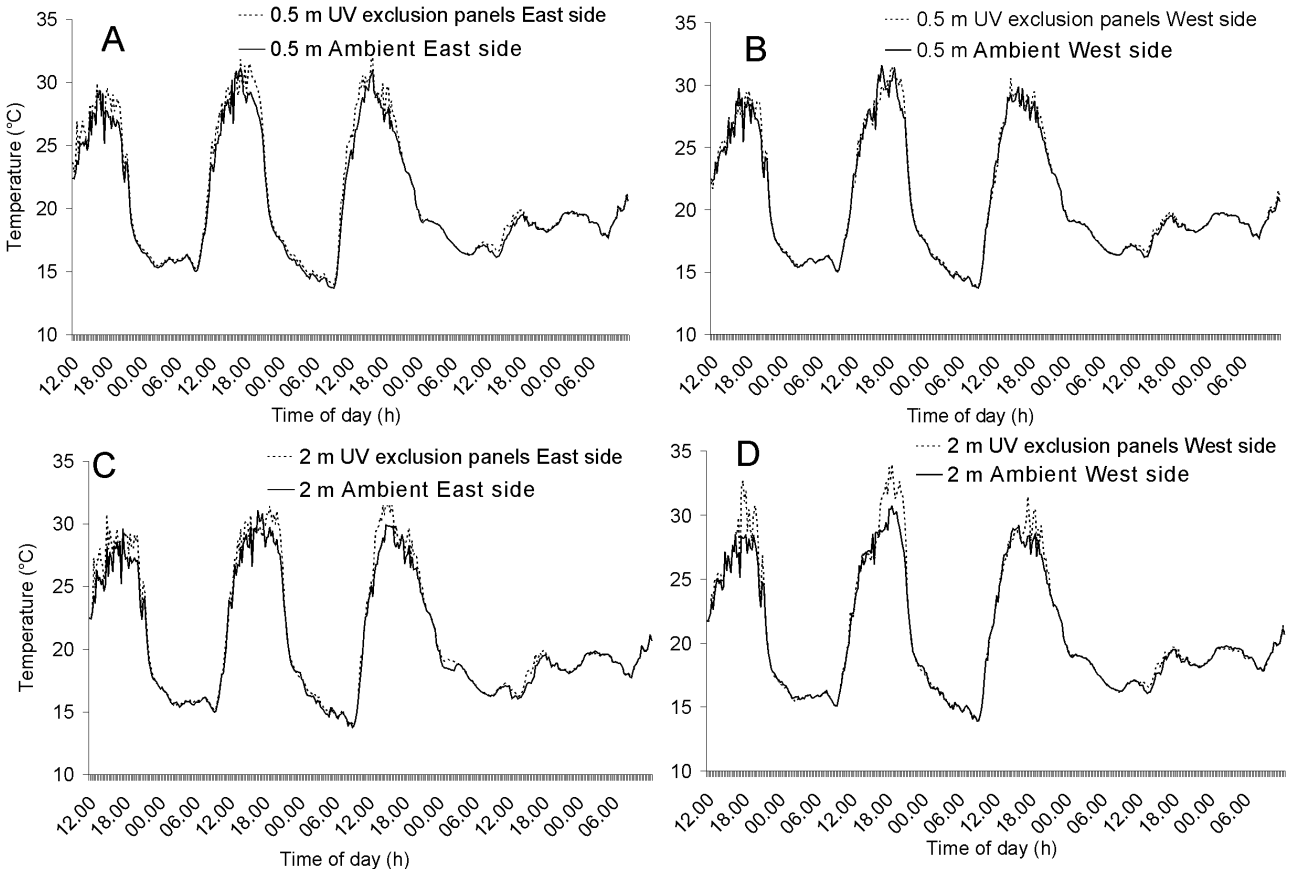

FIG. 2
Air temperatures at 0.5 m (Panels A, B) and at 2 m (Panels C, D) above ground-level on the East (Panels A, C) and West (Panels B, D) sides in the ultraviolet exclusion chamber, and under ambient conditions, in an apple orchard between 3 – 6 September 2004 (Apple Study 2).

TABLE I
Maximum quantum efficiency (F_v/F_m) and dark respiration (A_0) of fruit peel in five tropical fruits and the peel response ($\Delta F_v/F_m$ and ΔA_0) to 7 min exposure to 20 W m^{-2} UV radiation

Fruit Crop (cultivar)	F_v/F_m ($\Delta F_v/F_m$)	A_0 (ΔA_0) (μmol m^{-2} s^{-1})
Avocado ('Hass')	0.76 (0.0) NS	–5.4 (1.4)*
Custard apple ('African pride')	0.67 (0.0) NS	–6.8 (0.8)*
Macadamia ('847')	0.67 (0.0) NS	–12.3 (0.4)*
Pineapple ('Smooth cayenne')	0.71 (0.0) NS	–5.1 (0.9)*
Citrus ('Valencia')	0.45 (0.01) NS	–6.6 (0.5) *

NS, no significant difference ($P = 0.05$).
*, Significant difference ($P = 0.05$) based on a *t*-test.
Values in parentheses are the response at UV_{off} minus UV_{on}.

Pineapple, a crassulacean acid metabolism (CAM) plant, exhibited C_3 assimilation in the peel.

Apple studies

Study 1: A_0, $A_{1,000}$, F_v/F_m,and ΦII, or pigments data collected after 0, 5 and 10 d in the growth chamber, indicated no effect ($P = 0.05$) of the particle film (PF) treatments; therefore, data were pooled over the PF treatments at all sampling dates.

At 0 d in the growth chambers, peel F_v/F_m was unaffected by the UV filter, but the peel response beneath the UV filter showed a greater depression in $\Delta F_v/F_m$ than the control (Table II). Fruit grown beneath the UV filter had chlorophyll levels equivalent to those of the ambient trees, but reduced carotenoids and anthocyanins (Table II). There were no significant interactions.

After 5 d in the growth chambers, peel grown beneath the UV filter had the lowest F_v/F_m and the greatest $\Delta F_v/F_m$ (Table II) compared to the control treatments at both 28ºC and 40ºC. There was an interaction of growth chamber temperature with the UV filter treatment after 5 d in the growth chamber, in which peel grown beneath the UV filter had reduced carotenoid and anthocyanin levels (Table II) compared to ambient UV; and 40ºC day temperature further reduced the levels of anthocyanins and carotenoids in the UV filter treatment.

After 10 d in the growth chambers, F_v/F_m was lower and $\Delta F_v/F_m$ was greater for fruit grown beneath the UV filters. Carotenoids and anthocyanin levels were decreased in apple peel by the UV filter and 40ºC growth chamber temperature (Table II). There were no significant interactions.

Study 2: On the 21 June 2004 sampling, peel samples from fruit grown beneath the UV filter had a greater $\Delta F_v/F_m$ with reduced anthocyanins and carotenoids, compared to the control and PF treatments (Table III). ΔA_0 was significantly greater than 0, but there were no treatment effects. On the 27 July 2004 sampling, chlorophyll fluorescence and A_0 were unaffected by all the treatments. Anthocyanins and carotenoids were lowest for the UV filter treatment, and highest for the control, with the PF treatment being intermediate. ΔA_0 was significantly greater than 0, but there were no treatment effects. On the 26 August sampling, the PF treatment had the highest F_v/F_m response, but there were no differences in $\Delta F_v/F_m$ response to UV treatment. Anthocyanins were highest in the control and PF treatments, and lowest in the UV filter treatment. Carotenoids were highest in the control, lowest in the UV filter, and the PF treatment was intermediate. ΔA_0 was not significantly greater than 0 at this sampling time. Chlorophyll *a* and *b* and the xanthophyll pool declined over the three sampling dates, but did not demonstrate any treatment effect at any date, whereas carotenoid and anthocyanin levels increased over the three sampling dates. On all three sampling dates, there was no significant treatment effect on the xanthophyll (VAZ) pool; however, the trend was for the control and PF treatments to have higher xanthophyll contents than the UV filter treatment.

Study 3: $\Delta F_v/F_m$ was unaffected by UV treatment in 'Gold Rush' and 'Granny Smith' apple (Table IV). ΔA_0 increased in both cultivars with UV treatment.

DISCUSSION

The response of fruit peel A_0 to UV radiation was rapid (Figure 3) and was demonstrated in both tropical (Table I) and temperate fruits (Tables II–IV). UV radiation, and UV-B in particular, damages DNA (Britt, 1996) and the photosynthetic apparatus (Jansen *et al.*, 1998). The response of fruit peel to UV radiation was

TABLE II
Maximum quantum efficiency (F_v/F_m) and dark respiration (A_0), and chlorophyll, carotenoid, and anthocyanin pigment concentrations in 'Braeburn' apple peel grown with or without an ultraviolet (UV) filter in growth chambers

Growth Conditions	Chamber Temperature	UV Filter (–/+)	F_v/F_m ($\Delta F_v/F_m$)	A_0 (ΔA_0) (μmol m^{-2} s^{-1})	Chlorophyll *a* + *b* (mmol m^{-2})	Carotenoids (mmol m^{-2})	Anthocyanins (mmol m^{-2})
Ambient	Ambient	(–)	0.69 (0.04*) b†	–3.0 (0.0)	30	341 a	163 a
Ambient	Ambient	(+)	0.61 NS (0.09*) a	–2.1 NS (–0.1) NS	34 NS	168 b	84 b
5 d in growth chamber							
Growth chamber at 28 ºC	28ºC	(–)	0.70 a x (0.05*) b	–1.4 (-0.1)	26	253 a	139 a
Growth chamber at 28 ºC	28ºC	(+)	0.61 b (0.10*) a	–1.7 (–0.0)	23	189 b	94 b
Growth chamber at 40 ºC	40ºC	(–)	0.67 a (0.04*) b	–1.7 (0.0)	19	255 a	116 ab
Growth chamber at 40 ºC	40ºC	(+)	0.60 b (0.09*) a	–1.3 NS (–0.1) NS	21 NS	64 c	48 c
10 d in growth chamber							
Pooled	pooled	(–)	0.72 a (0.04*) b	–1.4 (0.1)	12	249 a	107 a
Pooled	pooled	(+)	0.61 b (0.09*) a	–1.5 NS (0.1) NS	13 NS	116 b	61 b
28ºC	28ºC	pooled	0.68 (0.07*)	–1.5 (0.2)	14	239 a	96 a
40ºC	40ºC	pooled	0.65 NS (0.06*) NS	–1.3 NS (0.0) NS	16 NS	126 b	37 b

NS, no significant difference ($P = 0.05$).
*, Significant difference ($P = 0.05$) based on a *t*-test of (UV_{off} minus UV_{on}) = 0.
†Values followed by different lower-case letters indicate a significant difference ($P = 0.05$) based on a protected Duncan's Multiple Range test by sample date.
Growth chambers were maintained at 28ºC or 40ºC. Data were collected after 0, 5, and 10 d. The peel received 7 min exposure to 20 W m^{-2} UV radiation (Apple Study 1).
Values in parentheses are the response ($\Delta F_v/F_m$ and ΔA_0) at UV_{off} minus UV_{on}.

TABLE III
Maximum quantum efficiency (F_v/F_m), dark respiration (A_0), and pigment concentrations in 'Gala' apple peel grown with or without ultraviolet (UV) filters and with a reflective kaolin particle film (PF) in the field season of 2004

Treatment	F_v/F_m ($\Delta F_v/F_m$)	A_0 (ΔA_0) (μmol m^{-2} s^{-1})	Chlorophyll *a* + *b* (mmol m^{-2})	Carotenoids (mmol m^{-2})	Anthocyanins (mmol m^{-2})	VAZ (mmol m^{-2})
21 June 2004						
Control	0.66 (0.01 b)[†]	–4.0 (0.9*)	20	103 a	84 a	18
PF	0.70 (0.03 b)	–3.8 (0.3*)	23	101 a	78 a	19
UV filter	0.71 NS (0.08* a)	–3.4 NS (0.2*) NS	19 NS	9 b	15 b	12 NS
27 July 2004						
Control	0.64 (0.02)	–1.6 (1.3*)	8	162 a	103 a	10
PF	0.66 (0.02)	–1.5 (1.5*)	10	100 ab	67 ab	9
UV filter	0.68 NS (0.01) NS	–1.8 NS (1.5*) NS	7 NS	21 b	22 b	6 NS
26 August 2004						
Control	0.61 b (0.00)	–2.2 b (0.1)	4	1383 a	567 a	3
PF	0.68 a (0.02)	–2.3 b (0.1)	3	993 ab	498 a	3
UV filter	0.65 ab (–0.02) NS	–1.6 a (0.2) NS	5 NS	357 b	180 b	2 NS

NS, no significant difference ($P = 0.05$).
*, Significant difference ($P = 0.05$) based on a *t*-test of (UV_{off} minus UV_{on}) = 0.
[†]Values followed by different lower-case letters indicate a significant difference ($P = 0.05$) based on a protected Duncan's Multiple Range test by sample date.
VAZ is the xanthophyll cycle pool size (i.e., violaxanthin plus antheraxanthin plus zeaxanthin).
The peel received 7 min exposure to 20 W m^{-2} UV radiation (Apple Study 2).
Values in parentheses are the response ($\Delta F_v/F_m$ and ΔA_0) at UV_{off} minus UV_{on}.

similar to the tissue responses of other species. Krause *et al*. (1999) demonstrated a decline in F_v/F_m in *Virola surinamensis* (Rol.) Warb. leaves within 10 min of UV treatment at approx. 42 W m^{-2}. Some algal species demonstrate reversible and increased A_0 with 2 W m^{-2} UV-B (Beardall *et al*., 1997); and 0.2 W m^{-2} UV-A inhibited alternative respiration, but stimulated CN-sensitive respiration (Mulley *et al*., 2001) with 1 – 6 h exposures. Increased dark respiration has been associated with moderate levels of UV radiation (Gwynn-Jones, 2001; Brandle *et al*., 1977; Sisson and Caldwell, 1976; Ziska *et al*., 1991) and was attributed to increased resource demands for protection and repair (Gwynn-Jones, 2001). All tropical fruit increased A_0 when challenged with UV, suggesting that UV repair mechanisms are induced and functioning with no adverse effect on photosystem II, since F_v/F_m and ΦII were unaffected. Tropical plants have evolved and have been domesticated under higher UV levels and, based on our findings, robust UV protection mechanisms are active in a range of tropical species. Krause *et al*. (1999) demonstrated that leaves of tropical plants can become fully protected against UV radiation, depending on their light acclimation and developmental stage. Tolerance of tropical species to UV was attributed to UV-absorbing substances, including anthocyanins, carotenoids, and VAZ, that increase with exposure in fully-illuminated leaves. Similarly, the photosynthetic rate of soybean leaves adapted to UV-B was unaffected by a UV-B challenge of approx. 0.5 W m^{-2}; however, the photosynthetic rate of non-adapted leaves was reduced by 20% (Mirecki and Teramura, 1984). UV radiation, in general, and UV-B in particular (Tevini and Teramura, 1989; Day and Neale, 2002) are documented to reduce net photosynthesis in leaves; however, $A_{1,000}$ was unaffected in all of our studies. Plant species would be expected to have effective repair mechanisms for their natural environment, and the moderate UV stress of 20 W m^{-2} at 40ºC did not exceed the repair capacity of plants, or cause irreversible damage.

TABLE IV
Maximum quantum efficiency (F_v/F_m) and dark respiration (A_0) in 'Gold Rush' and 'Granny Smith' apple peel in October 2004

Cultivar	F_v/F_m ($\Delta F_v/F_m$)	A_0 (ΔA_0) (μmol m^{-2} s^{-1})
'Gold Rush'	0.51 NS (0.01)	–2.4 (0.7*)
'Granny Smith'	0.43 NS (0.02)	–2.3 (0.7*)

NS, no significant difference ($P = 0.05$).
*, Significant difference ($P = 0.05$) based on a *t*-test of (UV_{off} minus UV_{on}) = 0.
The peel received 7 min exposure to 20 W m^{-2} UV radiation (Apple Study 3).
Values in parentheses are the response ($\Delta F_v/F_m$ and ΔA_0) at UV_{off} minus UV_{on}.

In contrast, 'Braeburn' apple had reduced F_v/F_m, with no increase in A_0 at all sampling dates. Solovchenko and Schmitz-Eiberger (2003) also demonstrated that the shaded peel of 'Braeburn' had reduced F_v/F_m with 11 W m^{-2} of UV radiation at room temperature. We suggest that apple fruit peel, developed under the UV filter, did not induce UV repair mechanisms to the same extent as did the ambient control. This finding is supported by the reduced F_v/F_m and increased $\Delta F_v/F_m$ on all three sampling dates in the UV filter treatment. However, in the presence of light, PSII and $A_{1,000}$ were unaffected by UV radiation (data not shown), suggesting that photosynthesis was able to supply the energy for UV repair mechanisms and the protection of PSII. Pfündel *et al*. (1992) demonstrated that UV-B effects were minimised by high PAR irradiation, and photo-reactivation was a key mechanism of DNA repair (Britt, 1996).

In contrast, the response of 'Gala' demonstrated a lack of $\Delta F_v/F_m$, except for the UV filter treatment, and increased ΔA_0 with UV treatment. The 'Gold Rush' and 'Granny Smith' responses also demonstrated no impact on $\Delta F_v/F_m$, with an increase in A_0.

'Braeburn' is more susceptible to sunburn than the other cultivars (Evans, 2004) and was the only cultivar that did not respond to UV with increased A_0, but with increased $\Delta F_v/F_m$, indicating PSII damage. The $\Delta F_v/F_m$ and A_0 responses were not correlated with pigment content (data not shown), indicating that other processes were involved in their response to UV treatment.

The PF treatments had no effect on F_v/F_m, A_0, $\Delta F_v/F_m$, or ΔA_0 in 'Braeburn' and 'Gala'. We hypothesise that the particle film, which reflects UV radiation (Glenn *et al*., 2002), might prevent UV adaptation in the fruit.

However, removal of the PF before measurement indicates that UV adaptation was occurring, since its response was not significantly different from the control receiving ambient UV radiation, but significantly different from the UV filter treatment.

There was a pattern in all the apple studies. When the $\Delta F_v/F_m$ response to UV treatment was < 0.02, ΔA_0 was generally > 0.4 μmol m^{-2} s^{-2} indicating a significant increase in dark respiration. Conversely, when the $\Delta F_v/F_m$ response to UV treatment was > 0.02, then ΔA_0 was < 0.4 μmol m^{-2} s^{-1}, indicating less stimulation of respiration (Figure 4). This pattern suggests that when UV repair mechanisms are effective, PSII is adequately protected with a $\Delta F_v/F_m$ of approx. 0, and protection occurs at the cost of higher respiration with ΔA_0 > 0.4 μmol m^{-2} s^{-1}. However, when the UV repair mechanisms are ineffective, not only is PSII damaged and $\Delta F_v/F_m$ > 0.02, but there is additional short-term damage to the repair mechanisms, indicated by a lack of respiration to provide energy. When fruit are not adapted to ambient UV levels, as in the UV filter treatments, there is greater damage to PSII, as measured by $\Delta F_v/F_m$ in the 'Gala' and 'Braeburn' studies. There was no significant correlation of $\Delta F_v/F_m$ or ΔA_0 with any of the pigments evaluated over all studies (data not shown), suggesting that other mechanisms are interacting with pigment content in the response of peel to UV radiation.

The technique of measuring fruit peel gas exchange responses to environmental conditions provides new insights into fruit physiology. Therefore, when fruits are exposed to new radiation conditions associated with Summer pruning and/or re-orientation from fruit growth that could cause SI, successful adaptation may be predicted by examining both the $\Delta F_v/F_m$ and ΔA_0 response.

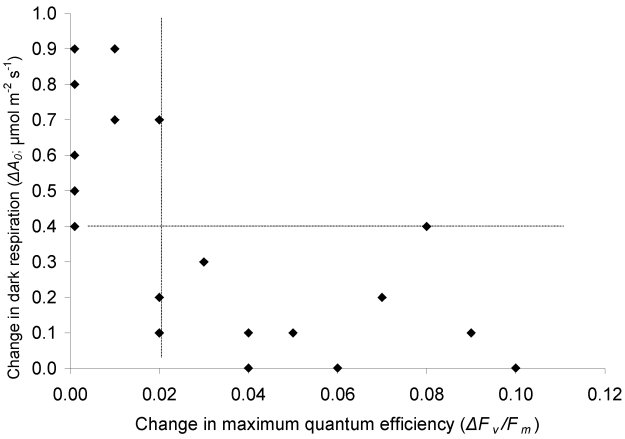

FIG. 4
Relationship of dark respiration (ΔA_0) in fruit peel with maximum quantum efficiency ($\Delta F_v/F_m$) when exposed to a 7 min treatment of 20 W m^{-2} ultraviolet (UV) radiation. Data (solid diamond symbols) are the difference in response between UV_{off} minus UV_{on} for treatment means in apple Studies 1, 2, and 3 (pooled data).

We thank Lailiang Cheng, Department of Horticulture, Cornell University for the analyses of xanthophyll cycle pool pigment concentrations in 'Gala' apple, unpublished information on carotenoid and xanthophyll cycle pigment patterns in apple, and a thoughtful review of the work. We also thank Leonardo Lombardini, Department of Horticulture, Texas A and M University, for his detailed review of the manuscript.

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